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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
SHUKLA, RAM R	

ART UNIT	PAPER NUMBER
1632	

DATE MAILED: 02/23/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/645,415	BERMUDES ET AL.	
	Examiner	Art Unit	
	Ram R. Shukla	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 October 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *2,5-14,16,26,40,49,52-61,63,100-188 and 2938* is/are pending in the application.

4a) Of the above claim(s) *See Continuation Sheet* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *2, 5, 6, 12, 14, 16, 26, 29, 30, 36, 38, 40, 49, 52, 53, 59, 61, 63, 105, 118, 131 and 144-155, 158-169 and 172-188* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *8/24/00* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *8-24-01*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

Continuation of Disposition of Claims: Claims withdrawn from consideration are 7-11,13,31-35,37,54-58,60,100-104,106-117,119-130,132-143,156,157,170 and 171.

DETAILED ACTION

1. Applicants' response filed 10-14-03 has been received.

2. Claims 3, 4, 27, 28, 50 and 51 have been cancelled.

3. New claims 142-188 have been entered.

4. As noted in the previous office action, an attenuated tumor targeted bacteria comprising an antiangiogenic factor as the primary effector molecule (derived from animal) and a bateriocin release factor as a secondary effector molecule was considered. Due to an advertant error claims 7, 8, 31, 32, 54 and 55drawn to a bacteriocin family member as the primary effector molecule were also included into the considered claims. Since this was not elected for prosecution these claims have been withdrawn.

5. Newly submitted claims 142, 143, 156, 157, 170 and 171 directed to an invention that is independent or distinct from the invention originally claimed for the following reasons: they are drawn to an invention that recites a bacteriocin family member as the primary effector molecule that was not elected for prosecution.

Since applicant has received an action on the merits for the originally presented invention, this invention has been constructively elected by original presentation for prosecution on the merits. Accordingly, claims 142, 143, 156, 157, 170 and 171 have been withdrawn from consideration as being directed to a non-elected invention. See 37 CFR 1.142(b) and MPEP § 821.03.

6. Claim 7-11, 13, 31-35, 37, 54-58, 60, 100-104, 106-117, 119-130, 132-141 remain withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected species. Election was made **without** traverse in Paper No. 21.

7. Claims 2, 5, 6, 12, 14, 16, 26, 29, 30, 36, 38, 40, 49, 52, 53, 59, 61, 63, 105, 118, 131 and 144-155, 158-169 and 172-188 drawn to an attenuated tumor bacteria comprising an anti-angiogenic factor as the primary effector molecule, an animal as the organism from which the primary effector molecule is derived and a

bacteriocin release factor as the secondary effector molecule are instant consideration.

Information Disclosure Statement

8. Applicants' submission of the IDS is acknowledged. Several reference that cite a NIH grant or an application have been crossed because they lack a publication date, are not available to the public and cannot be published in a patent.

Oath/Declaration/Specification

The objection to the specification and oath/declaration is withdrawn in view of the applicants' response and filing of a supplemental oath/declaration.

Claim Rejections - 35 USC § 112

9. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

10. Claims 2, 5, 6, 12, 14, 16, 26, 29, 30, 36, 38, 40, 49, 52, 53, 59, 61, 63, 105, 118, 131 and 144-155, 158-169 and 172-188 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claimed invention recites an attenuated tumor targeted bacteria that comprises one or more nucleic acid molecules encoding one or more primary effector molecules and one or more secondary effector molecules operable linked to

one or more promoters, wherein said attenuated bacteria is a facultative aerobe or facultative anaerobe.

In analyzing whether the written description requirement is met for genus claims, it is first determined whether a representative number of species have been described by their complete structure. When the claims are analyzed in light of the specification, instant invention encompasses genus of nucleic acid encoding anysecondary effector molecule. However, the specification only describes the structure of a nucleic acid encoding bacteriocin release factor (see the specification on page 40 and 41). While the specification discloses that the secondary effector molecule could be any molecule, the specification does not teach the structure of any other secondary effector molecule except bacteriocin release factor BRP.

Additionally, the claimed invention encompasses any nucleic acid encoding any angiogenic factor from any animal. While the specification provides a laundary list of the anti-angiogenic factors in pages 5-8, it does not teach the structure of the anti-angiogenic factors from a representative number of animals.

Next, then, it is determined whether a representative number of species have been sufficiently described by other relevant identifying characteristics (i.e. other than nucleotide sequence), specific features and functional attributes that would distinguish different members of the claimed genus. In the instant case, the only other identifying characteristic is that the secondary effector molecule will provide additional therapeutic value and/or facilitate the release of the contents of the modified bacterial vector (see page 41). However, the specification does not disclose any identifying characteristic as to how an artisan would have differentiated different members of the claimed genus. The specification dislxoses that the secondary effector molecule is proteinaceous or nucleic acid molecule, however the specification does not teach any characteristics of the proteins and nucleic acid encompassed by the claimed genus. Regarding the anti-angiogenic factors, the specification does not provide any description as to what were the identifying characteristics of the nucleic acids encoding the anti-angiogenic factors of different animals that would be representative of genus animals which is a large genus which would have subgenuses.

Accordingly, this limited information is not deemed sufficient to reasonably convey to one skilled in the art that the applicant is in possession of the broad genus of the modulators or agents at the time the application was filed. Thus it is concluded that the written description requirement is not satisfied for the claimed genus.

11. Claims 2, 5, 6, 12, 14, 16, 105, and 144-155 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for an attenuated Salmonella typhimurium wherein said attenuated Salmonella comprises a first nucleic acid encoding a primary effector molecule wherein said primary effector molecule is endostatin and a second nucleic acid encoding a secondary effector molecule weherein said secondary effector molecule is BRP, does not reasonably provide enablement for any attenuated tumor targeted bacteria comprising any number of nucleic acids encoding any number of primary effector molecules and any number of secondary effector molecules. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claims 26, 29, 30, 36, 38, 40, 118 and 158-169 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims 49, 52, 53, 59, 61, 63, 131 and 172-188 rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The invention of claims 2, 5, 6, 12, 14, 16, 105, and 144-155 recites an attenuated tumor targeted bacteria that comprises one or more nucleic acid

molecules encoding one or more primary effector molecules and one or more secondary effector molecules operable linked to one or more promoters, wherein said attenuated bacteria is a facultative aerobe or facultative anaerobe. Dependent claims recite a list of anti-angiogenic factors. Claims 26, 29, 30, 36, 38, 40, 118 and 158-169 recite a pharmaceutical composition whereas claims 49, 52, 53, 59, 61, 63, 131 and 172-188 recite a method of targeted delivery to tumor.

While determining whether a specification is enabling, one considers whether the claimed invention provides sufficient guidance to make and use the claimed invention, if not, whether an artisan would have required undue experimentation to make and use the claimed invention and whether working examples have been provided. When determining whether a specification meets the enablement requirements, some of the factors that need to be analyzed are: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and whether the quantity of any necessary experimentation to make or use the invention based on the content of the disclosure is "undue" (In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)). Furthermore, USPTO does not have laboratory facilities to test if an invention will function as claimed when working examples are not disclosed in the specification, therefore, enablement issues are raised and discussed based on the state of knowledge pertinent to an art at the time of the invention, therefore skepticism raised in the enablement rejections are those raised in the art by artisans of expertise.

The specification as filed is not enabling for the claimed invention commensurate with the scope of the claims because the specification does not sufficient guidance to make any attenuated bacteria comprising any number of nucleic acids encoding any number of primary and secondary effector molecules , treatment of a tumor with the bacteria and targeted delivery of the bacteria to a tumor. An artisan of skill would have required undue experimentation to make and use the claimed invention commensurate with the full scope of the claims because

the art of targeted delivery of gene and treatment of a tumor using attenuated bacteria was unpredictable and was not routine in the art.

The specification on page 10 discloses that genetically engineered Salmonella have been demonstrated to be capable of tumor targeting, possesses anti-tumor activity and are useful in delivering effector genes such as HSV TK to solid tumors (see lines 1-4). The specification, on pages 31-32 also, discusses Salmonella and its mutants as vector. The specification does not teach any other bacteria as vectors for delivery of effector molecules. Dietrich et al (Antisense & Nucleic Acid Drug Development 10:391-399, 2000) reviewed the state of the art of bacterial systems for the delivery of eukaryotic antigen expression vectors and noted that the four bacteria used as delivery systems were Salmonella typhimurium, Shigella flexmeri, E.coli and Listeria monocytogenes. Of these only Salmonella, Shigella and Listeria have been used in vivo in mice or rat and none of the bacteria have been used in humans (see table 1). Additionally, none of these were used for tumor targeted delivery, rather they were used vaccination. This clearly shows that at the time of the invention tumor targeted delivery of effector molecules in vivo in any animal, including humans was not routine and in the absence of teaching in the art of record, the specification has to teach making and using of any attenuated bacteria for effector molecule delivery in any animal including humans. However, the specification fails to provide enabling disclosure for the claimed invention. The method of producing an attenuated bacteria for in vivo delivery of effector molecules was unpredictable at the time of the invention because a rational approach to design a live attenuated bacterial vaccine involves genetic modification of the bacterial pathogen to make the pathogen less virulent while maintaining the stability of protective antigen expression that provides immune protection. The attenuation should be an inherent property of the bacterial vaccine and not be dependent on fully functional host defenses and immune response capabilities (Curtiss R. J. Clin. Invest. 110(8):1061-1066, 2002). In addition, the attenuation of a bacterium requires the modification of specific bacterial genes that render the bacterial strain non-virulent. For example, inactivation of PhoP/phoq regularoty system in S. typhi results in strains, which are suitably attenuated

for use as vaccines (Tiball et al Vaccine 19:4175-4184, 20001, see page 4177 sec 3.1). Furthermore, the development of live attenuated bacterial vaccine has not been always predictable. For example, development of a live attenuated *Shigella* vaccine that is sufficiently attenuated to be non-reactive yet adequately invasive to be highly immunogenic took 30 years in making, since it required substantial understanding of molecular genetic basis of virulence of *Shigella* (Curtiss page 1063, col.2). The specification fails to disclose what are the bacterial regulatory systems in these bacteria, mutation of which would result in the making of a live attenuated bacterial strain that would provide protect a mammal against any specific bacterial infection. The specification does not provide any guidance to make any attenuated bacteria for tumor targeted delivery. It is emphasized that the art of record while teaches attenuated Shigella and Salmonella for vaccination, does not teach making attenuated bacteria for tumor targeting.

The specification on pages 81-84 teaches construction of Salmonella expressing endostatin, in vitro expression of endostatin by the Salmonella and intravenous administration of the Salmonella to C57bl/6 mice transplanted with Colon 38 tumor fragment (see page 83, figure 17). While the specification describes tumor inhibition, there is no evidence that the Salmonella vector was delivered only to the tumor and not to any other tissue. Therefore, the specification does not provide any evidence that the Salmonella expressing endostatin was targeted to the tumor only and not to other tissues.The claimed invention encompasses treatment of tumor by targeting expression of any ant-angiogenic factor to the tumor which would require that the claimed attenuated bacteria when administered by any rout would reach the tumor and the nucleic acid encoding the anti-angiogenic protein(s) would be expressed in the tumor in sufficient quantity to treat the tumor. It is noted that claims recite a laundry list of anti-angiogenic factors, for example in claim 6. It is noted that while an artisan could make recombinant nucleic acids that could express the listed anti-angiogenic factors recited in the claims, there is no evidence that all these molecules could be expressed at level sufficient to treat a tumor or these factors could treat a tumor.

Additionally, claimed invention recites nucleic acid encoding any anti-angiogenic protein from any animal, however, neither the specification teaches structure of the nucleic acid encoding a representative number of factors nor does it teach how to make and use the nucleic acid and any attenuated bacteria comprising them.

Given the lack of guidance or direction provided by the instant specification into would have required undue experimentation to use the claimed attenuated bacteria for *in vivo* gene delivery and treatment of tumor in any animal including humans. It is emphasized that while there is evidence for vaccination using Salmonella or Sighella there is no evidence for targeted delivery of a nucleic acid encoding an anti-angiogenic protein in a tumor. For bacteria to function as DNA delivery systems into mammalian cells in general or in a human in particular, the bacteria must first enter the cell and then escape from the vacuole to the cytosol. Movement from the vacuole to the cytosol is unpredictable because in many instances the bacteria are lysed by the host cell's defense system and any plasmids carried by the bacteria are degraded preventing expression of heterologous nucleotide sequences. At best it would appear that only a few cells, if any may be transformed with plasmid DNA carried by a bacterial vehicle as Grillot-Courvalin (Nature Biotechnology, 1998, 16: 862-866) suggest that "direct introduction of DNA from bacteria to mammalian cells has been reported in very few instances". See page 865, starting with the first line of the discussion. Grillot-Courvalin support such observations by reporting that "factors such as entry route may have an effect" on DNA delivery. Grillot-Courvalin go on to report that a mouse dendritic cell line, which can internalize bacteria via micropinocytosis, did not express incoming DNA at 24 hours post-transfer. Grillot-Courvalin suggest that this failure could reflect rapid degradation of the invading bacteria by this cell type. It would appear that use of bacteria as DNA delivery vehicles is not very efficient in other cell lines as well as Grillot-Courvalin have reported that *E.coli* carrying a nucleotide sequence encoding the green fluorescent protein are only able to transform 0.3-1% of a transfected macrophage cell line. See the paragraph bridging pages 864-865. These observations are corroborated by Dietrich et al (Nature Biotechnology, 1998, 16: 181-185) who report that only about 0.03% of macrophages infected with a

mutated form of *Listeria monocytogenes* express a green fluorescent protein reporter gene. See page 183, column 2. Dietrich et al also suggest that expression of a heterologous nucleotide sequence is not stable over time by observing a gradual loss of fluorescence over time. See page 183 at the bottom of column 2. Dietrich report that the low efficiency of expression of GFP as compared to the number of macrophages infected may be due to the fact that "only some of the attenuated bacteria infecting the host cells survive the antimicrobial milieu inside the phagosome and are able to escape into the host cell cytosol, whereas the others are totally digested, including the plasmid DNA and that not all listeriae being taken up reach the host cell cytosol as an intact viable entity, but the plasmid DNA is still released into this compartment. See page 184 at the top of column 2.

Claimed invention encompasses delivery by administering the bacteria by any route, however, it is not routine in the art to administer the bacteria by any route. While the art teaches administration by oral route, the issues of unpredictability regarding antigen stability and antigen expression at a level sufficient to induce an immune response abound. A general issue of unpredictability of oral vaccines is the poor immunogenicity displayed by most antigens when given orally. See Pascual et al (Behring Inst. Mitt., 1997, 98: 143-152) on page 143. Pascual et al report that there are several issues compounding the development of live oral bacterial vaccine vectors, including the fact that there is a "lack of a well tolerated, highly immunogenic bacterial vector for use in humans". See page 144. While the instantly claimed invention is not directed to vaccination and is directed to tumor directed delivery, the issues of unpredictability discussed above will be applicable in the instant case.

The state of the art as evidenced above suggests that use of bacteria as a vehicle for transferring heterologous nucleotide sequences to eukaryotic cells of an animal is undeveloped, inefficient, and unpredictable. The studies recited above demonstrate that only low efficiency of reporter gene expression occurs in cell lines *in vitro* and only contemplate that bacteria could be used to transfer heterologous DNA sequences to the cells of an organism supporting the Examiner's assertion that use of

bacteria to transfer DNA *in vivo* is undeveloped and unpredictable. Given, the unpredictable and undeveloped nature of the state of the art, the lack of working examples provided by the specification it would have required undue experimentation to make and use the claimed bacteria commensurate with full scope of the claims.

12. No claim is allowed.

13. Examiner's Comments:

It is noted that while the art of record teaching making of an attenuated Salmonella comprising a nucleic acid encoding a protein that is a primary effector molecule and use of agents that would facilitate the lysis of the bacteria to release the nucleic acid encoding the primary effector molecule, the art of record does not teach an attenuated Salmonella comprising a nucleic acid encoding a primary effector molecule and a nucleic acid encoding a secondary effector molecule, both in the same attenuated Salmonella.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ram R. Shukla whose telephone number is (571) 272-0735 . The examiner can normally be reached on Monday through Friday from 7:30 am to 4:00 p.m. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Deborah Reynolds, can be reached at (571) 272-0734. The fax phone number for TC 1600 is (703) 703-872-9306. Any inquiry of a general nature, formal matters or relating to the status of this application or proceeding should be directed to the William Phillips whose telephone number is (571) 272-0548.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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